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Echinostome cercariae from *Biomphalaria straminea* (Mollusca: Planorbidae) in a ricefield from northeastern Argentina

Echinocercarias de *Biomphalaria straminea* (Mollusca: Planorbidae) en un campo de arroz del noreste de Argentina

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Abstract. The species of larval Echinostomatidae that infect *Biomphalaria straminea* (Dunker, 1848) in a ricefield in Corrientes province, Argentina, were studied. Examination of 5 510 snails during 2 rice cultivation cycles, from December 2010 to May 2011 and from December 2011 to April 2012, revealed the presence of 3 new species: *Echinocercaria* sp. XIII, *Echinocercaria* sp. XIV and *Echinocercaria* sp. XVI in 36 snails (0.65%). The most common species was *Echinocercaria* sp. XVI. Prevalence of 3 species during the first rice cultivation cycle was low (< 1%), whereas during the second rice cultivation cycle it was somewhat higher, with prevalence greater than 1% only in *Echinocercaria* sp. XVI. The species of echinocercariae in *B. straminea* from the agricultural habitat described in the present study are new additions to the species already reported for the genus *Biomphalaria* in the region.

Key words: Digenea, freshwater snails, larval stages, agricultural wetlands, Argentina.

Resumen. Se estudiaron las especies de echinocercarias que infectan a *Biomphalaria straminea* (Dunker, 1848) en un campo de arroz de la provincia de Corrientes, Argentina. La prospección de 5 510 caracoles durante 2 ciclos de cultivo de arroz, desde diciembre de 2010 a mayo de 2011 y desde diciembre de 2011 a abril de 2012, reveló la presencia de 3 nuevas especies: *Echinocercaria* sp. XIII, *Echinocercaria* sp. XIV y *Echinocercaria* sp. XVI en 36 caracoles (0.65%). La especie más común fue *Echinocercaria* sp. XVI. Durante el primer ciclo de cultivo de arroz las prevalencias de las 3 especies fueron bajas (< 1%), mientras que durante el segundo ciclo de cultivo de arroz las prevalencias fueron algo mayores, con valores superiores al 1% solo en *Echinocercaria* sp. XVI. Las especies de echinocercarias descritas en el presente estudio parasitando a *B. straminea* de un ambiente agrícola, se adicionan al registro de especies para el género *Biomphalaria* en la región.

Palabras clave: Digenea, caracoles de agua dulce, estadios larvales, humedales agrícolas, Argentina.

Introduction

In South America, some snail species of genus *Biomphalaria* Preston, 1910 are intermediate hosts of *Schistosoma mansoni* Sambon, 1907. In Brazil, the American country most affected by this parasite, its natural intermediate hosts are *B. glabrata* (Say, 1818), *B. tenagophila* (d'Orbigny, 1835) and *B. straminea* (Dunker, 1848), in that order of importance (Bezerra et al., 2003; Thiengo and Fernandez, 2007; Lambertucci, 2010).

Although the presence of this parasite has not yet been reported in Argentina, the geographical range of the endemic schistosomiasis areas in Brazil has been expanding to the state of Rio Grande Do Sul, adjacent to northeastern Argentina (Graeff-Teixeira et al., 1999, 2004), an area where 2 of the natural vectors in Brazil, the snails *B. tenagophila* and *B. straminea*, are common species (Rumi et al., 2008).

Previous studies on the fauna of larval trematodes in planorbid molluscs of genus *Biomphalaria* (*B. occidentalis* Paraense, 1981, *B. tenagophila*, *B. orbignyi* Paraense, 1975, *B. peregrina* (d'Orbigny, 1835) and *B. straminea*) have been carried out in natural environments of Corrientes province, northeastern Argentina (Ostrowski-de Núñez et al., 1990, 1991, 1997; Hamann et al., 1991) but there is little information concerning agroecosystems such as ricefields (Rumi and Hamann, 1990; Fernández et al., 2013). These

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latter environments, which provide favorable conditions for the development of dense populations of planorbids, are important from the health perspective due to frequent human direct contact with the water (Rumi, 1986). In turn, Corrientes province is the main rice producer of Argentina, with more than half of its cultivated area occupied by rice crops (Aacrea, 2003).

On the other hand, several studies have demonstrated that some species of echinostome and amphistome larvae may interfere with the natural resistance of the snail to *S. mansoni* infection (Lie et al., 1977a, b; Adema et al., 2000; Silva Garcia et al., 2010; Spatz et al., 2012). In this sense, it is essential to continue the study of trematode larval species that infect freshwater snails of genus *Biomphalaria*, especially those echinostome species that may affect the interaction between *S. mansoni* and its host before the possible introduction of *S. mansoni* in the area. Therefore, the goals of the present paper are to report and describe new species of echinostome cercariae from the freshwater molluscs *B. straminea* in a ricefield from the Corrientes, Argentina.

Materials and methods

Study area. The study site was an agricultural area of 25 ha, with 4 cultivated rice parcels connected or associated to the Paraná river basin; the area is located approximately 30 km south from Corrientes city, in Corrientes province, Argentina (27°40'23.5" S; 58°48'21.6" W). During the sampling months, water depth ranged between 5 and 10 cm in the cultivated parcels, and between 10 and 50 cm in the irrigation canals. Water temperature ranged between 17° C and 28° C in the first rice cultivation cycle and between 18° C and 30.5° C in the second rice cultivation cycle.

In the initial phase of flooding, no vegetation was observed in the irrigation canals; later on, the predominant hydrophilic vegetation consisted of *Sagittaria montevidensis* Cham. and Schlecht, *Ludwigia peploides* (Kunt) P.H. Raven, *Hydrocotyle ranunculoides* L.fil., and *Limnobium* sp. During the months of sampling several waterbird species were observed: *Egretta thula* (Molina, 1782), *Ardea alba* Linnaeus, 1758, *Nomonyx dominicus* (Linnaeus, 1766), *Jacana jacana* (Linnaeus, 1766), *Vanellus chilensis* (Molina, 1782), *Himantopus mexicanus* (Statius Müller, 1776), *Aramus guarauna* (Linnaeus, 1766), *Mycteria americana* Linnaeus, 1758, *Tringa flavipes* Gmelin, 1789 and *Plegadis chihi* (Vieillot, 1817).

Sampling and laboratory procedure: snails were collected during 2 rice cultivation cycles in the flooding period, from the time of sowing to soon after harvesting of the rice, between December 2010 and May 2011, and between December 2011 and April 2012. Five samplings were carried out in each rice cultivation cycle. The samples were taken manually by 2 persons who sampled during 1 hour and a half from the cultivated parcels and irrigation canals, using simple mesh nets locally known as "copos" (25 cm frame diameter). In the laboratory the snails were kept individually in vials with 20 ml of tap water, and were observed for the emergence of cercariae. Apparently uninfected snails were dissected to check for other larval intramolluscan stages (e. g., immature infections and metacercariae). Cercariae were studied alive, with and without vital dyes. Drawings were made using a camera lucida attached to a Carl Zeiss Jena microscope. Cercariae fixed in hot 4% formalin were preserved in vials with 70% ethanol, and deposited in the Helminthological Collection of the Centro de Ecología Aplicada del Litoral, Corrientes, Argentina. Photographs were taken with a Leica DFC 295 camera mounted on a Leica DM 2500 microscope. Specimens studied by scanning electron microscopy were dehydrated in an ethanol series, dried using the critical point technique, coated with gold-palladium and examined with a Jeol 5800 LV Scanning Electron Microscope. Measurements of heat-killed and formalin-fixed specimens are expressed in micrometers (μm), with range followed by the mean ± SD in parentheses. The "open nomenclature" recommended by Odening (1971) was adopted for new species of cercariae. For counts of the number of collar spines the criteria given by Kanev et al. (2009) was followed.

To determine the second intermediate hosts, 3 laboratory-reared larval specimens of *Physalaemus albonotatus* (Steindachner, 1864) and 4 laboratory-reared specimens of *Serrapinnus piaba* (Lütken, 1875), collected from an artificial tank were exposed to emerged cercariae. The amphibian larvae and fishes were maintained in small aquaria under controlled conditions until dissection, which was carried out 12-69 hours post-exposure (PE).

Results

A total of 5 510 snails were examined (2010-2011: n= 3 494; 2011-2012: n= 2 016), 36 of which (0.65%) were infected with echinostome species. During the first rice cultivation cycle the prevalence of infection ranged between 0.02% (*Echinocercaria* sp. XIII) and 0.06% (*Echinocercaria* sp. XVI), and during the second rice cultivation cycle the prevalence of infection ranged between 0.10% (*Echinocercaria* sp. XIV) and 1.39% (*Echinocercaria* sp. XVI) (Fig. 1). The most common species was *Echinocercaria* sp. XVI. The shell size of infected snails ranged from 5.00 to 12.50 (mean= 8.12 ± SD= 1.58) in the first rice cultivation cycle and from